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Interaction of alcohol and hepatitis viral proteins: implication in synergistic effect of alcohol drinking and viral hepatitis on liver injury

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Abstract

Alcohol drinking and viral hepatitis are both recognized as major causes of liver disease worldwide, and they frequently coexist and synergistically cause liver injury in patients with chronic liver disease. Several mechanisms have been implicated in exacerbation of liver injury in patients with alcohol drinking and viral hepatitis. These include impairment of host defense and liver regeneration by alcohol consumption. The findings obtained from my laboratory have demonstrated that alcohol potentiates cooperatively several signals activated by hepatitis B virus X protein (HBX) or hepatitis C virus core protein, and HBX sensitizes hepatocytes to tumor necrosis factor-alpha (TNF-α)– and ethanol-induced apoptosis by a caspase-3-dependent mechanism, which may also contribute to the synergistic effect of alcohol drinking and viral hepatitis on liver injury.

Keywords: Alcohol; Viral hepatitis; Liver injury; Hepatitis C virus core protein; Hepatitis B virus X protein; NF-kappa B

Alcohol drinking and viral hepatitis are both recognized as major causes of liver disease worldwide, and they frequently coexist in patients with chronic liver disease (Mendenhall et al., 1993; Nevins et al., 1999; Regev & Jeffers, 1999; Schiff, 1999). For example, it has been reported that 14%–38% of patients with alcoholic liver disease were detected positively with anti-hepatitis C virus (HCV) antibody and HCV RNA (Mendenhall et al., 1993). Epidemiological findings have established that alcohol consumption and viral hepatitis act synergistically to promote the development and progression of liver disease (Brechot et al., 1982; Khan & Yatsuhashi, 2000; Kondili et al., 1998; Nevins et al., 1999; Ohta et al., 1998; Wiley et al., 1998). For example, viral hepatitis with alcohol consumption is associated with the accelerated progression of liver injury (Nevins et al., 1999; Ohta et al., 1998), as well as with a higher frequency of cirrhosis (Kondili et al., 1998; Wiley et al., 1998) and a higher incidence of hepatocellular carcinoma (HCC) (Brechot et al., 1982; Khan & Yatsuhashi, 2000) than that observed with viral hepatitis alone or with alcohol consumption alone. The mechanism underlying the interaction of alcohol and hepatitis virus, however, remains largely unknown. Several mechanisms have been proposed on the basis of clinical observations. First, ethanol inhibits interferon-α antiviral response (Mochida et al., 1996; Nguyen et al., 2000; Ohnishi et al., 1996; Okazaki et al., 1994) and promotes viral replication (Sawada et al., 1993). Second, ethanol consumption impairs host defense by decreasing inflammatory response, altering cytokine production, and inducing abnormal reactive oxygen intermediate generation (Cook, 1998; Szabo, 1999). Third, chronic ethanol consumption inhibits liver regeneration (Duguay et al., 1982; Wands et al., 1979). Recent findings obtained from my laboratory (Kim et al., 2001) support the suggestion that cooperative activation of several signals by ethanol and hepatitis viral proteins may also contribute to the synergistic effect of alcohol drinking and viral hepatitis on liver disease.

Hepatitis C virus is an RNA virus with a genome size of about 10 kb, which encodes a number of structural (core, E1, E2, and p7) and nonstructural (NS2, NS3, NS4A, NS4B, and NS5B) proteins (Choo et al., 1991; Di Bisceglie, 1998). Kato et al. (2000) have investigated the effects of these proteins on intracellular signaling and demonstrated that HCV core protein has the strongest influence on several intracellular signals, especially nuclear factor-kappa B (NF-κB)–, activator protein-1 (AP-1)–, and serum response element (SRE)–associated pathways. Hepatitis B virus (HBV) is a partially double-stranded DNA virus with a circular genome that is 3.2 kb, which encodes four proteins: two nonstructural proteins (X and polymerase) that are involved in viral replication and two structural proteins (core protein and surface protein) (Ganem & Varmus, 1987). Among these four proteins,

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the protein (HBX) encoded by X gene has been shown to activate many signaling pathways, such as AP-1-, activator protein-2 (AP-2)–, NF-κB-, and SRE-associated pathways (Andrisani & Barnabas, 1999). It is believed that activation of these signaling pathways by HBX or HCV core proteins plays an important role in the progression of liver injury, cirrhosis, and HCC (Andrisani & Barnabas, 1999). For example, AP-1 is a heterodimeric complex containing products of the *jun* and *fos* oncogene families and is implicated in cell proliferation and tumor formation (Hsu et al., 2000). Activation of AP-1 has been implicated in HBV-associated hepatocarcinogenesis (Lauer et al., 1994). NF-κB is a ubiquitous transcription factor that is activated by a wide variety of cytokines, mitogens, viruses, and free radicals (Pahl, 1999). Activation of NF-κB has been implicated in hepatic inflammation, liver regeneration, liver injury, and HCC (Baeuerle, 1998; Cressman et al., 1994; Tai et al., 2000). My colleagues and I have investigated the effects of ethanol on HBX- or HCV core protein–mediated activation of NF-κB in hepatic cells (Kim et al., 2001).

Our findings show that ethanol, HBX, or HCV core protein alone markedly activates NF-κB in primary mouse hepatocytes (Kim et al., 2001). Treatment of primary hepatocytes with combination of ethanol and HBX or HCV core protein causes further additive but not synergistic induction of NF-κB. Furthermore, we provide several lines of evidence that support the suggestions that ethanol potentiation of HBX or HCV core protein activation of NF-κB requires ethanol metabolism. First, acute ethanol exposure (6–12 h) inhibits basal and HBX- or HCV core protein–mediated activation of NF-κB in HepG2, a cell line that expresses very low or no ethanol metabolism enzymes. Second, acute ethanol exposure (6–12 h) induces about 2.7-fold activation of basal levels of NF-κB and additively, but not synergistically, potentiates HBX- or HCV core protein–activated NF-κB in E47 cells, a HepG2 cell line that overexpresses CYP2E1. Third, blocking of ethanol metabolism by 4-methylprazole attenuates ethanol alone- or ethanol plus HBX–induced NF-κB activity. Finally, acetaldehyde, the major ethanol metabolite, additively potentiates HBX- or HCV core protein–activated NF-κB in HepG2 cells, E47 cells, and primary hepatocytes. The effects of chronic ethanol consumption on HBX- and HCV core protein–mediated activation of NF-κB were also examined (Kim et al., 2001). Chronically (8 weeks of) feeding mice with ethanol results in a sixfold increase in CYP2E1 protein expression as well as an increase in the basal levels, HBX, or HCV core protein activation of NF-κB. Taken together, our findings seem to indicate that ethanol additively potentiates HBX- or HCV core protein–mediated activation of NF-κB in the liver, and ethanol metabolism is required for such potentiation (Kim et al., 2001).

Many agents can activate NF-κB, and most of them do so through a common pathway that involves activation of NF-κB-inducing kinase (NIK) and consequent I-κB kinase (IKK) and I-κB. We have demonstrated that the NIK–IKK–I-κB signaling cascade is also involved in HBX-, HCV core protein–, or acetaldehyde-activated NF-κB (Kim et al., 2001). First, transfection with dominant negative NIK, IKK, or I-κB constructs attenuates HBX-, HCV core protein–, or acetaldehyde-activated NF-κB. Second, acetaldehyde rapidly activates NF-κB binding and I-κB phosphorylation, followed by degradation of I-κB. Finally, acetaldehyde activates IKK1 and IKK2 in HepG2 cells. Zhu et al. (1998, 2001) have shown that HCV core protein binds the cytoplasmic domain of tumor necrosis factor (TNF) receptor 1 (TNFR1) in vitro, enhances Fas-associated death domain (FADD)–mediated apoptosis, and suppresses TNF receptor-associated death domain (TRADD) signaling of TNF receptor. This seems to indicate that TNF receptor may be involved in HCV core protein–mediated activation of NF-κB. Our findings show that HCV core protein–mediated activation of NF-κB, AP-1, and AP-2 is completely abolished in the hepatocytes isolated from TNFR1 (−/−) mice, supporting the suggestion that TNFR1 is essential for HCV core protein–mediated signals in vivo (Kim et al., 2001). Similarly, HBX-mediated activation of NF-κB, AP-1, and AP-2 is also completely abolished in the hepatocytes isolated from TNFR1 (−/−) mice, supporting that HBX targets TNFR1 complex (Kim et al., 2001). Taken together, our findings seem to indicate that both HBX and HCV core proteins target TNFR1 in the liver. Clinical study findings also support the suggestion that TNFR1 is active in patients with viral hepatitis. First, serum TNF-α levels and expression of TNF-α in the liver are dramatically elevated in patients with viral hepatitis (Gonzalez-Amaro et al., 1994; Nelson et al., 1997). Second, several signals (e.g., NF-κB) activated by TNF-α in the liver are elevated in patients with viral hepatitis (Tai et al., 2000). Increasing evidence seems to indicate a critical role of TNFR1-mediated signaling in development and progression of alcoholic liver injury. First, TNF-α antibody has been shown to attenuate alcohol-induced liver injury (Iimuro et al., 1997). Second, alcoholic liver injury has been shown to be abolished completely in TNFR1-deficient mice, providing solid evidence in support of the hypothesis that TNFR1-mediated signaling plays an important role in the development of alcohol-induced liver injury (Yin et al., 1999). Therefore, targeting of TNFR1 by HBX or HCV core protein could contribute to cooperative effects of alcohol drinking and viral hepatitis on liver disease, and NF-κB could be a potential therapeutic target to prevent liver injury caused by alcohol drinking and viral hepatitis.

The HCV core protein has been shown to sensitize hepatic cells to TNF-α-induced apoptosis (Zhu et al., 1998). We show that primary hepatocytes obtained from HBX transgenic mice are more susceptible to ethanol- or TNF-α-induced apoptotic killing, as demonstrated by DNA fragmentation, flow cytometry, and nuclear condensation (B. Gao et al., unpublished observations, 2001). Ethanol and TNF-α treatment induces more oxidative stress, activation of caspase-3, and activation of poly(ADP-ribose) polymerase in primary hepatocytes obtained from HBX transgenic mice than in primary hepatocytes of control mice.

Blocking of caspase-3 antagonizes ethanol- and TNF-α-induced apoptotic killing in primary hepatocytes obtained from HBX-transgenic mice (B. Gao et al., unpublished observations, 2001). Thus, our findings seem to indicate that HBX sensitizes primary mouse hepatocytes to ethanol- or TNF-induced apoptosis, which may be implicated in the synergistic effect of ethanol drinking and viral hepatitis on liver disease.

In summary, the findings obtained from my laboratory have demonstrated that ethanol potentiates HBX- or HCV core protein–mediated activation of NF-κB in the liver, and HBX sensitizes hepatocytes to TNF-α- and ethanol-induced apoptosis. The future directions of my and my colleagues' research are to study the interaction of ethanol and other hepatitis viral proteins and the effects of ethanol on liver injury on transgenic mice with hepatitis viral proteins.

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